

X-ray Structure of a Novel Endolysin Encoded by Episomal Phage phiSM101 of *Clostridium perfringens*

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1 Introduction

Gram-positive bacteria possess a thick cell wall composed of a mesh polymer of peptidoglycans, which provides physical protection. Endolysins encoded by phages infecting bacteria can hydrolyze peptidoglycans in the bacterial cell wall, killing the host bacteria immediately. The endolysin (Psm) encoded by episomal phage phiSM101 of enterotoxigenic *Clostridium perfringens* type A strain SM101 exhibits potent lytic activity towards most strains of *Clostridium perfringens*. Psm has an N-terminal catalytic domain highly homologous to *N*-acetylmuramidases belonging to the glycoside hydrolase 25 family, and C-terminal tandem repeated bacterial Src homology 3 (SH3_3) domains as the cell wall binding domain. In this study, the X-ray structure of Psm was determined to elucidate the cell wall recognition mechanisms of Psm [1].

2 Experiment

The recombinant Psm was expressed in *E. coli* BL21-CodonPlus-RIL as a protein histidine-tagged at the N-terminus, and purified by nickel-charged immobilized metal affinity chromatography and anion-exchange chromatography. Crystals of Psm for data collection were obtained in a droplet containing 1.5 μ l of the protein solution (11.5 mg/ml) and 1.5 μ l of the reservoir solution (2 % (w/v) PEG3350, 15 % tacsimate) against 80 μ l of the reservoir solution. X-ray diffraction data were collected using ADSC Quantum 210r or 270 CCD detectors on PF-AR NW12A and NE3A (PF, Tsukuba, Japan), at 100 K. The initial phase for Psm was determined by a molecular replacement method, using the structure of 'cellosyl', *Streptomyces coelicolor* lysozyme (PDB: 1JFX), as a search model. Structure was refined to R-factor of 0.228.

3 Results and Discussion

The structure of Psm can be divided into three domains; the catalytic domain (Met1–Ile202) and tandem-repeated SH3_3 domains (SH3N domain; Asp210–Leu275 and SH3C domain; Gln276–Leu343) (Fig. 1). The catalytic domain adopts an irregular (β/α)₅ β ₃ barrel structure, in which 6th and 7th β -strands (b6 and b7) are connected not by an α -helix but by a long loop, and 8th β -strand (b8) is anti-parallel to other β -strands. The proposed catalytic residues (Asp13 and Glu103) are located at the C-terminal side of the β -barrel to form the catalytic site. The SH3N and SH3C domains have 51 %

amino acid sequence identity, and their structures are almost equivalent with the root-mean-square deviation of main chain atoms of 0.5 Å. Each of these has a distorted β -sheet by seven β -strands and two short $_3$ helices. The SH3N and SH3C domains are arranged in an approximate 2-fold symmetry without a linker region. A typical SH3 domain recognizes a peptide ligand using a shallow groove formed by hydrophobic amino acid residues. The SH3N and SH3C domains also have shallow grooves on the molecular surface, in which an acetic acid (ACT) and malonic acid (MLA) are bound. When Psm binds to the bacterial cell wall, tandem repeated SH3_3 domains are expected to recognize the peptide side chains of peptidoglycans to assist the catalytic domain hydrolyzing the glycan backbone.

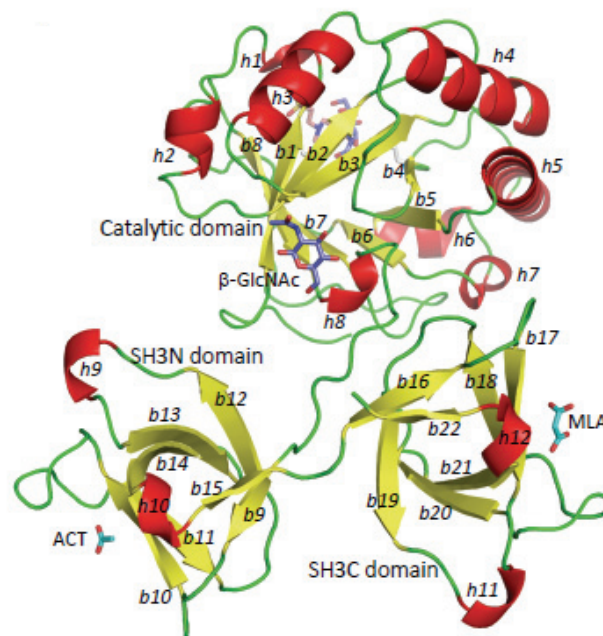


Fig. 1: The overall structure of Psm.

Acknowledgement

We thank the PF staff for support of data collection.

References

[1] E. Tamai *et al.*, *Mol. Microbiol.* **92**, 326 (2014).

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